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The effect of RanBPM on the regulation of the hypothalamic–pituitary axis by thyroid hormone receptors is isoform-specific

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Abstract

Although crucial for TH homeostasis, the molecular mechanisms responsible of thyroid hormone receptors (TRs)-mediated regulation of the hypothalamic–pituitary–thyroid axis (HPT) axis remain unclear. We examined the role played by TR-isoforms in combination with RanBPM, a novel coactivator of TRs. In transient transfections studies with the human TRH and TSH-α subunit promoters, we found that the overexpression of RanBPM increases the transcriptional activity of all TR-isoforms by a magnitude of 1.7- to 3-fold. The addition of RanBPM, in the absence of THs, increased the ligand-independent activation (LIA) of TRα1 and TRβ1 on both promoters tested by 300% and 200%, respectively, whereas, the LIA of TRβ2 was not significantly modified. This data reinforces the concept of isoform-specific regulation of genes of the HPT axis and demonstrates that RanBPM may be an important factor to achieve adequate regulation of nTREs in the presence of low TH levels.

Keywords: Steroid receptors; Thyroid hormone receptors; Thyrotropin (TSH); Thyrotropin-releasing hormone (TRH); Coactivator; RanBPM

Thyroid hormone receptors (TRs) are nuclear receptors that function as ligand-dependent transcription factors through the recognition of thyroid hormone response elements (TREs) in the promoter region of target genes ([1], for review). These genes have been reported to be either activated or repressed in the presence of thyroid hormone (TH). Genes that are activated by TH contain positive TREs (pTREs) and have been more extensively studied: ligand-dependent modifications of TR surfaces result in the selective recruitment of corepressors inducing gene silencing in the absence of ligand and their replacement by coactivators in the presence of T_3.

Thyroid hormone homeostasis is carefully regulated by a negative feedback system, the hypothalamic–pituitary–thyroid (HPT) axis, which includes the thyrotropin-releasing hormone (TRH) secreted by neurons in the supraoptic and supraventricular nuclei of the hypothalamus and the thyrotropin (TSH)-α and -β subunits secreted by the thyrotroph cells located in the anterior pituitary ([2], for review). TRs activate the transcription of those negatively regulated TREs (nTREs) in the absence of ligand and induce gene repression in the presence of TH [3,4]. As for pTREs, coregulator proteins are involved in the regulation of nTREs but different mechanisms are implicated ([5], for review).

In humans, two genes encode thyroid hormone receptors to produce TR-isoforms: TRα1, TRα2, TRβ1, and TRβ2 [1]. In the HPT axis, the TR-isoforms are not equally expressed: studies have shown that although TRα1 is present, only TRβ1 and TRβ2 are highly expressed in the hypothalamus and anterior pituitary [6,7]. Given the particular expression profile of the TRβ-isoforms, cellular models, and transgenic mice studies have shown their unique ability to regulate the TRH and TSH subunits genes compared to the TRα-isoform [8–11]. The functional specificity of the TRβ-isoforms in the regulation of nTREs resides in their

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specific A/B domain located in the amino-terminal portion [9,11,12]. This region is thought to contribute to differential interactions with coregulators which result in their more potent ability to regulate nTREs [8,9].

We have recently identified RanBPM as a novel coactivator of TRs [13] that stimulates the transcriptional activity of different pTREs and binds more strongly to the TRβ-isoforms. We thus hypothesized that RanBPM could also influence the regulation of the genes involved in the HPT axis. We found that, as for pTREs, RanBPM activates the transcription of the nTREs studied and that the effect of RanBPM is TR-isoform-specific.

Materials and methods

Plasmid constructions. The expression plasmids used were pcDEBΔ-RanBPM90 [14], pSG5-TR-isoforms (human cDNA) [9], and the corresponding empty vectors as controls. The negative thyroid response element (nTRE) reporter constructs included the 5′-flanking sequences from the human TRH (−900/+55) [4], or the common glycoprotein α-subunit (TSH-α, 846/+26) downstream of a minimal thymidine kinase promoter and fused to the luciferase gene in the PA3 vector [15].

Cell culture and transient transfections. JEG-3 cells (*Homo sapiens*, ATCC HTB-36) were maintained in minimum essential media (MEM) supplemented with 10% foetal bovine serum (FBS) (Invitrogen, Burlington, ON). The day before transfection, cells were seeded in 6-well plates at approximately 10,000 cells per well. Cells were transfected using the calcium phosphate precipitate technique (CellPhect, Amersham Biosciences, Que., Canada) [13,16]. Into each plate, 500 ng of TR-pSG5, 3 μg of RanBPM-pcDEBΔ or the corresponding empty vectors, and 10 μg of the luciferase reporter gene were used. Sixteen hours after transfection, cells were fed with fresh media supplemented with charcoal and resin stripped FBS with the addition of T_3 (10 nM) or the vehicle. Cells were harvested 24–36 h following the hormonal-treatment and processed for luciferase assays. Luciferase activity was measured using an EG&G Berthold lumat LB 9507 luminometer. Data are from at least three independent experiments performed in triplicate, and are displayed as means ± SEM. Statistical analysis was performed using unpaired Student's *t*-tests with the SigmaStat program version 2.03.

Results

Effect of RanBPM on the regulation of the human TRH promoter

To investigate the role of RanBPM on genes negatively regulated by THs we studied the transcriptional activity of the human TRH promoter in luciferase gene reporter assays. These were carried out in JEG-3 cells, a well established cellular model to investigate negatively regulated genes by THs due to the endogenous expression of the 9-*cis* retinoic acid receptor (RXR), the heterodimerization partner of TRs [15,17]. Furthermore, JEG-3 cells express low levels of functional TRs [17] allowing the study of the differential transcriptional activity of each TR-isoform.

The influence of RanBPM on the regulation of the TRH promoter is depicted in Fig. 1. In Fig. 1A, results are represented in relative luciferase units (RLUs: photons emitted by each sample normalized on the control). In the absence of RanBPM our results show that the TRβ-isoforms, especially TRβ2, are more potent than TRα1 to stimulate the

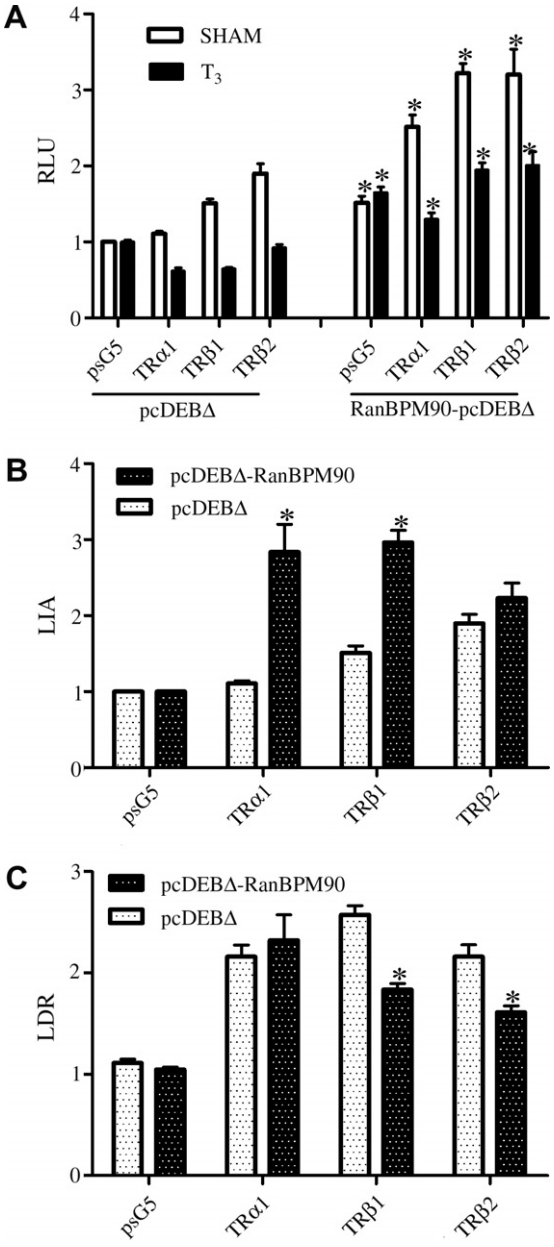

Fig. 1. RanBPM increases the transcriptional activity of TRs on the human TRH promoter. JEG-3 cells were transiently transfected with the TRH promoter region −900/+55 fused to a luciferase reporter gene, TRs-pSG5 and RanBPM90-pcDEBΔ or the corresponding empty vectors. (A) The results are represented in relative luciferase units (RLU: photons emitted by each sample, normalized on the control, pSG5/pcDEBΔ, in the absence of T_3). RanBPM90 increases the transcriptional activity of TRs in the presence ($^*p < 0.001$) and the absence of T_3 ($^*p < 0.05$) compared with the empty vector (pcDEBΔ). (B) The effect of RanBPM on TR ligand-independent activation (LIA: RLUs of TR in the absence of T_3/RLUs of the vector alone in the absence of T_3). Overexpression of RanBPM generates a significant increase of the LIA of TRα1 and TRβ1 compared to the empty vector, $^*p < 0.005$. (C) The ligand-dependent repression (LDR: RLUs in the absence of T_3/RLUs in the presence of T_3). The overexpression of RanBPM caused a significant decrease of the LDR of TRβ1 and TRβ2, $^*p < 0.005$. Data are from at least three independent experiments performed in triplicate and are displayed as means ± SEM.

expression of the TRH promoter in the absence of T_3, as previously reported [9–11,17]. Overexpression of RanBPM